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TITLE: Dissecting Androgen-Dependent and Independent Signaling Pathways Using RNA Interference-Based Functional Genomics in Human Cells

PRINCIPAL INVESTIGATOR: Isil Guney, Ph.D.

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute
Boston, MA 02115

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Isil Guney, Ph.D.

E-Mail: isil_guney@dfci.harvard.edu

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14. ABSTRACT

We had previously identified androgen responsive genes in LNCaP prostate cancer cells using microarray technology. I have performed a high throughput loss of function screen using RNA interference (RNAi) in order to identify androgen responsive genes that are critical for androgen induced proliferation. I am currently investigating whether the genes identified in my screen function in prostate tumor progression. At the same time, in collaboration with scientists from the Broad Institute of Harvard and MIT, we screened by RNAi LNCaP cells among a panel of human cancer cell lines to identify synthetic lethal partners of oncogenic KRAS, and identified TBK1 as a gene that is essential in cells with mutant KRAS. The results from this study are currently in review for publication.

15. SUBJECT TERMS
Prostate cancer, RNAi, functional genomics, androgen signaling

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Introduction:

Prostate cells depend on a crucial level of androgenic stimulation for proliferation and survival. Although hormone therapy is the mainstream treatment for prostate cancer, the targets of androgen receptor signaling crucial for prostate cell growth have not been elucidated. The identification of androgen responsive genes and pathways that are critical for proliferation will shed light into the mechanism of androgen action, and yield insight into how cancer cells subvert regulation of controlled proliferation and become refractory to hormone manipulation. Using microarray technology, we had previously identified 58 androgen responsive genes in LNCaP human prostate cancer cells. In this project, I have conducted a loss of function screen in order to identify androgen responsive genes that are critical for androgen induced proliferation and also function in prostate cancer progression, with the objective of discovering novel therapeutic targets for the treatment of prostate cancer.

Given that many known oncogenes have proven to be challenging therapeutic targets, an alternative approach to direct targeting of known cancer alleles is to identify genes whose suppression selectively impair the viability of cells harboring an oncogenic allele. In collaboration with scientists from the Broad Institute, we screened by RNAi LNCaP prostate cancer cells among a panel of human cancer cell lines in order to identify synthetic lethal partners of oncogenic KRAS,

Body:

Task 1: Perform a loss of function screen to identify genes critical for androgen receptor stimulated cell proliferation

I have conducted a high throughput RNA interference screen in LNCaP cells to ablate the expression of androgen regulated genes. 300 short hairpin RNAs were used in the screen and were delivered into cells by lentiviral infection. PSA production and proliferation were monitored to identify androgen target genes critical for proliferation (Figure 1). Some of the genes that have scored in both the PSA production and the proliferation assays have been validated (Figure 2).

Proliferation

Ranking #	shRNA #	Z score	
1	238	-2.04358262	fli
2	15	-1.9927052	primase
3	41	-1.93425357	
4	55	-1.72476613	c-maf
5	142	-1.72083589	
6	14	-1.71598933	primase
7	242	-1.71401911	
8	144	-1.71340008	NM_023078
9	57	-1.60752595	c-maf
10	233	-1.5909379	clecsf12
11	237	-1.55619949	fli
12	245	-1.55613389	
13	37	-1.54607488	
14	157	-1.54605651	acaca
15	264	-1.5437582	AR
16	83	-1.54045956	
17	56	-1.53146117	c-maf
18	102	-1.52228734	maguk
19	166	-1.49715269	abcc4
20	78	-1.48321925	
21	222	-1.48285431	
22	285	-1.47643278	
23	247	-1.40205587	
24	178	-1.35147852	odz1
25	125	-1.34326435	
26	165	-1.34295582	abcc4
27	111	-1.27647673	NM_018081
28	30	-1.25221369	
29	254	-1.25075943	
30	112	-1.24126944	NM_018081
31	263	-1.23886258	AR
32	158	-1.23723709	acaca
33	176	-1.20657276	odz1

PSA

Ranking #	shRNA #	Z score	
1	238	-2.00120233	fli
2	15	-1.93077739	primase
3	41	-1.90604766	
4	242	-1.66981732	
5	14	-1.66377084	primase
6	55	-1.66104829	c-maf
7	142	-1.65487237	NM_023078
8	144	-1.62384325	NM_023078
9	233	-1.56680068	clecsf12
10	264	-1.55129102	AR
11	237	-1.54537979	fli
12	57	-1.5324615	c-maf
13	102	-1.5215086	maguk
14	37	-1.50553134	
15	245	-1.50449086	
16	83	-1.49537023	
17	56	-1.47464551	c-maf
18	222	-1.46231418	
19	157	-1.45724105	
20	78	-1.4196147	
21	285	-1.41369496	
22	166	-1.40970603	abcc4
23	247	-1.36839171	
24	165	-1.28352793	abcc4
25	178	-1.27301629	
26	111	-1.25493973	NM_018081
27	229	-1.24749359	clecsf12
28	263	-1.24446441	AR
29	125	-1.22879595	
30	112	-1.223623	NM_018081
31	254	-1.20515475	

FIGURE 1: Androgen target genes critical for proliferation. Short hairpin RNAs (shRNA) targeting the expression of androgen regulated genes were delivered into LNCaP cells by lentiviral infection. Cell proliferation and PSA production were monitored using luminescence based Cell Titer Glo assay and ELISA assay, respectively, to identify androgen target genes critical for proliferation. shRNAs that reduced proliferation and PSA production to at least 1 standard deviation below the mean are shown. The Z score represents the number of standard deviations the observed phenotype is relative to the mean. Genes that scored in both of the assays are also indicated.

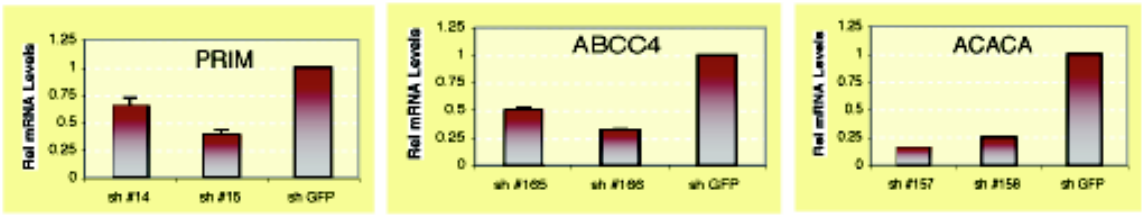

FIGURE 2: Ability of shRNAs to suppress target gene expression. The efficiency of gene silencing by each of the shRNAs that scored in the proliferation and PSA screens is being assayed by real-time PCR in triplicate to monitor correlation with functional phenotype. Relative mRNA levels are shown where expression was normalized to control GFP hairpin. Data from three representative assays are shown.

I also conducted a loss of function RNAi screen LNCaP cells as a part of a larger effort to screen 17 human cancer cell lines in order to identify genes whose suppression selectively affects the viability of cancer cells harboring oncogenic KRAS. Using a lentiviral library consisting of 5002 shRNAs targeting 957 genes encoding kinases and cancer related genes, we found that the suppression of the kinase TBK1 consistently induced apoptosis in cell lines with mutant KRAS (data not shown).

Task 2: Study the relevance of the validated genes in tumor progression and its possible role in the development of androgen independent prostate cancer

I have overexpressed the genes identified in Task 1 in immortalized and transformed prostate epithelial cells that my lab had previously generated (1). I have also overexpressed the genes in androgen dependent LNCaP and LAPC4 cells. I have conducted *in vitro* proliferation and soft agar assays to determine whether the genes play a role in prostate cancer progression (Figure 3, data not shown). I will be conducting *in vivo* xenograft experiments to corroborate the *in vitro* data.

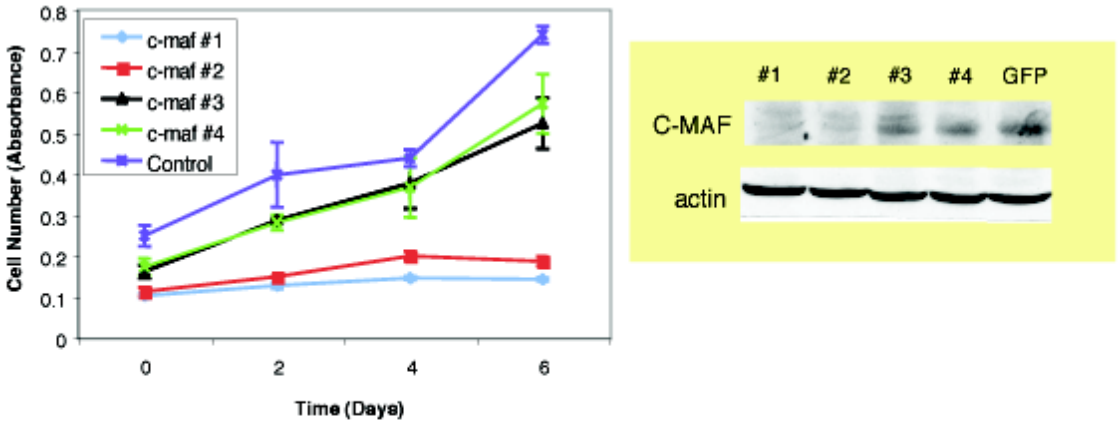

FIGURE 3: In vitro proliferation following C-MAF RNAi. LNCaP cells were infected with shRNAs targeting C-MAF and proliferation was monitored over six days using the luminesence based Cell Titer Glo assay. Immunoblotting was performed to monitor efficiency of gene silencing .

Key Research and Training Accomplishments:

1- Optimized conditions for conducting a high throughput screen in LNCaP cells.
2- Conducted an RNAi screen in LNCaP cells.
3- Identified a synthetic lethal partner of oncogenic KRAS.
4- Acquired significant expertise in manipulating high throughput genomic tools.

Reportable Outcomes:

Once I identify androgen induced genes that function in prostate cancer progression, I will write a manuscript describing the results for publication. The manuscript describing

TBK1 as a synthetic lethal partner of oncogenic KRAS has been submitted for publication.

Conclusions:

My studies have identified a set of androgen regulated genes that are critical for androgen mediated proliferation. I am continuing to investigate whether these genes function in prostate tumor progression. I expect that I will achieve my objective of identifying genes that mediate androgen induced proliferation that also play a critical role in prostate cancer. In collaboration with scientists from the Broad Institute, my studies have also identified TBK1 as a therapeutic target in KRAS mutant cancers.

References:

1- Berger et al. (2004) “Androgen-induced differentiation and tumorigenicity of human prostate epithelial cells.” Cancer Res 64(24):8867-75.